

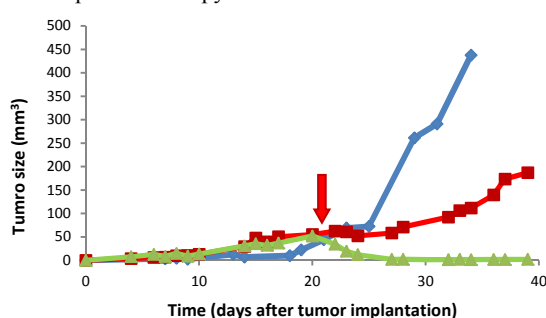
# Predicting response to hyperbaric oxygen radiotherapy treatment in high grade gliomas using Magnetic Resonance Imaging techniques

Nuria Arias<sup>1</sup>, Jesús Pacheco-Torres<sup>1</sup>, and Pilar López-Larrubia<sup>1</sup>  
<sup>1</sup>Biomedical Research Institute, CSIC, Madrid, Madrid, Spain

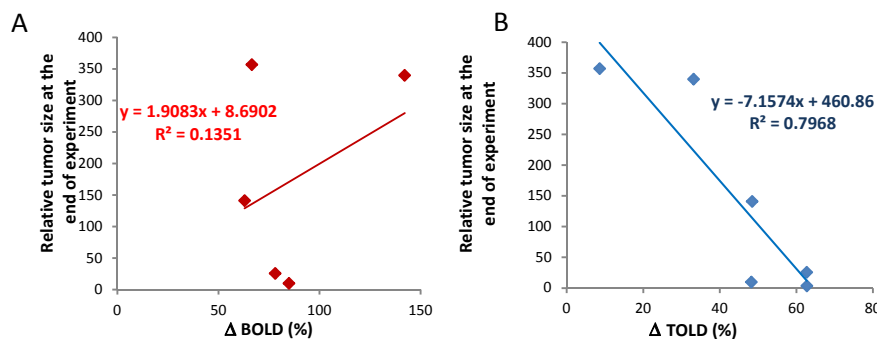
**Introduction.** Several studies indicate a relationship between tumor hypoxia and tumor aggressiveness, poor outcome (1, 2) and resistance to therapies (3, 4). Tumor hypoxia results from the negative balance between the oxygen demands of the tissue and the capacity of the vasculature to deliver satisfactory oxygen provisions. Due to a misregulation of angiogenic processes, neoplastic tissues often show hypoxic areas heterogeneously distributed in the tumor mass. There are several methods to measure hypoxia, but none of them have the desired requirements for routine clinical application (5). Due to its special characteristics, Magnetic Resonance Imaging (MRI) is an interesting alternative to measure tumor oxygenation. Several treatments have been specifically developed to modulate hypoxia in order to improve therapy success (photodynamic therapy, breathing a gas with high oxygen content during radiotherapy...). However, it would be desirable to know *a priori* those tumors sensitive to these modulation and, thus, that could benefit from these types of treatments. BOLD (Blood Oxygenation Level Dependent) contrast, based on T2\* changes induced by deoxyhemoglobin, is sensitive to vascular oxygenation and blood flow. Recently, several studies have shown a way to measure tissue oxygenation level based on T1 shortening of water due to concentration of molecular oxygen in the tissue (6). These images have been called TOLD (Tissue Oxygenation Level Dependent) (5)

**Methods.** High grade gliomas were induced in Wistar rats (200-220 g) by stereotaxic injection of C6 cells in the right caudate nucleus. MRI evaluations were carried out in a horizontal 7T system (Bruker Pharmascan®) with a <sup>1</sup>H selective birdcage resonator of 38 mm. Animals were anesthetized with 2% isoflurane in oxygen, placed in a heated probe and physiologically monitored. To measure tumors volume, T2-weighted (T2W) and T1-weighted (T1W) images with contrast agent (Magnevist®, Bayer) were acquired at different times after cells injection. BOLD and TOLD contrast images were acquired the day previous to radiation treatment. A series of interleaved TOLD (T1W) and BOLD (T2\*W) images were acquired during transition from air (baseline) to oxygen breathing. 12 animals with tumors were studied: 6 were irradiated while breathing pure oxygen (hyperoxia) and 6 while breathing air (normoxia). Treatment was performed in a biologic irradiator (Shepherd Mark J-30 800 Ci <sup>137</sup>Cs) using 10 Gy doses. Therapy success was assessed by measuring the relative tumor volume at the end of the experiment compared to the tumor size the day previous to the radiation. MRI images were analyzed with Image J (NIH, USA) software. Tumor changes in TOLD and BOLD were normalized respective to the changes in contralateral hemisphere ( $\Delta$ BOLD or  $\Delta$ TOLD)

**Results.** Figure 1 shows how different tumors evolve with therapy. Tumors treated in normoxic conditions (blue in figure 1) stop growing immediately after irradiation but, after three or four days, they grow again. On the other hand, tumors treated in hyperoxic conditions (green in figure 1) decrease their tumor size. There are also tumors treated in hyperoxic conditions that behave similarly to normoxic ones (red line in figure 1). Similar tumor volumes can depict completely different responses. We can establish that  $\Delta$ BOLD response has not relation with the relative increase of growth volume (figure 2A) at the end of the experiment. Nevertheless,  $\Delta$ TOLD depict an inverse relationship with tumor size at the end of the experiment (figure 2B). Tumors showing  $\Delta$ TOLD bigger than 40% respond well to therapy whereas tumors depicting  $\Delta$ TOLD smaller than 40% do not respond to therapy.



**Fig. 1.** Tumor size change with time after implantation. Red arrow indicates the day of radiation. Blue line: normoxic conditions, red and green lines: hyperoxic conditions



**Fig. 2.** Relative tumor size (% of the tumor size the day before treatment in hyperoxic conditions) at the end of the experiment based on their  $\Delta$ BOLD (A) and  $\Delta$ TOLD (B) response

**Discussion.** In this study, we propose BOLD and TOLD MRI acquisitions to detect those tumors that would improve their response to radiotherapy by breathing a gas with high oxygen content during radiation. These results are in good agreement with previous data in prostate tumor lines (7,8). Since these measurements are entirely non-invasive, they appear worthy of further exploration and correlation with response to other therapies.

**Bibliography.** [1] Evans SM, Koch CJ. *Cancer Lett.* 2003;195(1):1-16. [2] Padhani AR, Krohn KA, Lewis JS, Alber M. *Eur. Radiol.* 2007;17(4):861-872. [3] Vaupel P, Mayer A, Briest S, Hockel M. *Adv. Exp. Med. Biol.* 2005;566:333-342. [4] Brizel DM, Dodge RK, Clough RW, Dewhirst MW. *Radiother. Oncol.* 1999;53(2):113-117. [5] Pacheco-Torres J, Lopez-Larrubia P, Ballesteros P, Cerdan S. *NMR Biomed* 2011;24(1):1-16. [6] Matsumoto K, Bernardo M, Subramanian S, Choyke P, Mitchell JB, Krishna MC, Lizak MJ. *Magn Reson Med.* 2006;56(2):240-246. [7] Bourke VA, Zhao D, Gilio J, Chang CH, Jiang L, Hanh EW, Mason RP. *Int. J. Radiat. Oncol. Biol. Phys.* 2007;67:1179-1186. [8] Zhao D, Jiang L, Mason RP. *Methods Enzymol.* 2004;386:378-418.